The Effect of Sampling Time

on Blood Lactate Concentration ([Bla]) in Trained Rowers

ORIGINAL INVESTIGATION

by

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ABSTRACT

Purpose: To compare blood lactate concentration ([Bla]) at 15s and 45s during the 1 minute rest period between each stage of an incremental test in rowers and to establish the validity of using interchangeable sampling times. ***Methods:*** Seventeen male club rowers (mean ± SD, age 28.8 ± 5.7 years, height 186.9 ± 5.1 cm, body mass 85.4 ± 6.6 kg) performed an incremental rowing ergometer test, consisting of 5 stages of 4 corresponding to approximately 80% HR_{max} . A 10 microlitre earlobe blood sample was collected from each subject at 15 s and again at 45 s in the final minute of each test stage and analysed in duplicate. A maximum of 10 s was allowed for blood collection. *Results*: Statistical analysis using limits of agreement and correlation indicated a high level of agreement between the two [Bla] samples for all five test stages (agreement >95%, confidence intervals [CI] = -0.5 to 1.5, r=0.97, p<0.05). *Conclusion*: These results suggest that a sampling time between 15 s and 45 s may be recommended for the valid assessment of the [Bla] threshold in rowing performance monitoring. This extends the current sampling time of 30 s used by physiologists and coaches for National and club level Rowers. ***Key words:*** incremental protocol, blood lactate concentration, sampling time, limits of agreement.

PURPOSE

Blood lactate concentration ([Bla]) response to exercise can accurately predict endurance performance and is commonly used as a measure of endurance fitness[1,2,3,] . Although there is an abundance of information on [Bla] in response to exercise, the effect of training on the [Bla] threshold[4,5] and site specifity[6,7,8] and validity of Bla analysers,[9,10,11] there is scant research examining the protocols used for boat and ergometer based [Bla] testing and specifically the rationale behind the selected blood sampling time.

Sub-maximal [Bla] testing protocols are used as a regular component of fitness testing in National and club level rowing. Most of the protocols currently used are discontinuous, resulting in up to 5 blood samples being collected within a sub-maximal test and should give adequate data to identify the rower's [Bla] threshold and help in the prescription of a suitable individualised training programme.

However, there are many problems associated with [Bla] testing on a boat in the water. In particular; i) the ability of the coach/exercise physiologist to collect a [Bla] sample within the 30 s time period commonly suggested within the rowing community (British International Rowing Organisation, Olympic Medical Institute), and ii) the process of cross referencing [Bla] values determined in-boat with those using rowing ergometry when blood collection timings are essential. Bourdin *et al*[12] determined that [Bla] elicited during an incremental graded exercise test in the laboratory did not differ significantly from that measured during an on-water training session with intensity determined from heart rate corresponding to 2-3 $mmol.l^{-1}$. However, this study did not look at sampling times or lactate kinetics, particularly at the higher and lower ranges of lactate concentrations. Sampling time is the length of time allocated for blood collection from which blood lactate concentration is measured. The rate of appearance, clearance and exchange of blood lactate determines the lactate concentration, the kinetics of which form an integral part in the

decision on the length sampling time can be before it no longer reflects the physiological status of the subject at the relevant stage.

At present, there is no general consensus as to how long the sampling interval can be in order to minimise variability in [Bla] while making collection more practical by optimising the sampling time available for blood collection.

The aim of this study is to investigate the variability in [Bla] during an incremental rowing exercise test by i) comparing [Bla] collected at a sampling time of 15 s and 45 s at each work stage and ii) assessing the impact this difference in sampling time may have on subsequent determination of the lactate threshold. If agreement between the two sampling times are high then valid recommendations for future testing may be proposed.

METHODS

Subjects

Seventeen male heavy weight rowers, (mean±SD) mass 85.4±6.6kg, age 28.8±5.7years, height 186.9±5.1cm, participated in this study. All subjects were volunteers from rowing clubs in the South East of England. Inclusion criteria for the study included weight category (>72 kg) and ability to perform a sub 7-min 2000m row (6.30±0.2min) on a Concept 2 rowing ergometer (Concept 2 Ltd, Wilford, Nottingham, UK).

Each subject was thoroughly informed of the procedure and any associated risks before providing their written consent to participate in the study and full University ethics approval was obtained prior to testing. Pre-testing criteria included; no training for 24 hours, no consumption of food for 4 hours and to avoid alcohol, caffeine and/or carbohydrate-rich beverages, 24 hrs prior to testing. Adequate hydration was encouraged through consumption of water only.

Experimental Design and Procedures

One investigator collected all measurements throughout the trial. Heart rate (HR) was measured continuously during each test using a telemetric system (Polar Accurex, Polar Electro Oy, Kempele, Finland) and [Bla] was analysed from a 10-µl sample of capillary blood collected from a shallow earlobe puncture whilst the subject remained seated at rest in the rowing ergometer. Whole blood was analysed in duplicate using the Biosen 5040 Eppendorf [Bla] analyser (Eppendorf-nether-hinz, GMBH, Hamburg, Germany) reliability > 98.5% at 12mmol.$l^{-1.}$

The sub-maximal incremental step test

Subjects warmed-up for 10-min at a workload corresponding to <90bpm (~ 45%HRmax) followed by a short interval of stretching. This relatively low workload was considered appropriate to avoid any lactate accumulation that may confound subsequent results. A drag co-efficient of 130 was set on the ergometer providing equal resistance to all subjects.

The incremental protocol consisted of five stages of 4 min duration. The workloads (watts) of the test were determined from each subject's previous best time for a 2000m row on an ergometer. From this we calculated the 500 metre split time and a further 10 s was added to this time so that the subject did not reach their maximum power and speed during the five stages of the test. For example:- subject A with a personal best of 6.06min for a 2000m row on the ergometer would have a split time of 1 min 31s (6.06 min = 366 s divided by number of 500 metre splits in a 2000 metre race (4) = 366/4 = 91.5 s which = 1min 31s) + 10s. This time was converted to the corresponding wattage for the 5^{th} stage of the test using the Avicon Power Conversion table as supplied by the British Olympic Medical Institute (2004). Workloads 4 to 1 were calculated by deducting 25 watts for each stage respectively.

Whole blood was collected at 15 s and 45 s during a 1 min rest interval following each workload. A maximum of 10 s was allocated for blood collection and any sample collected outside this period was deemed void. A total of 3 subjects' data were excluded due to void samples. After the 1-min rest interval, the subject resumed rowing at the next highest wattage (stage 2). This procedure was repeated for each progressive workload.

Further analysis was undertaken to evaluate the impact that the extension of sampling time may have on subsequent training programmes based on HR training zones. This was achieved using a 15 s and 45 s lactate curve plotted against time to determine any difference in the subjects' heart rate at lactate threshold.

Statistical Analysis

Initial analysis used Pearson's Product-Moment Correlation to observe the correlation between 15 s and 45 s samples but not to determine agreement. This will show the strength of the relationship between the two variables, which are expected to be high as they are from the same subjects on the same test. Agreement between the two sampling times, which will give an indication of whether the two sampling times can be used interchangeably, were undertaken using Bland and Altman[13] 95% limits of agreement (LoA). These were calculated between [Bla] at 15 s and 45 s for each of the five stages and for the sum of all stages. From this, homogeneity of variance could also be demonstrated.

Paired two-sample for mean t-tests were undertaken to calculate P values and to determine the difference between [Bla] at 15 s and 45 s for each stage and across all five stages as one group. The level of statistical significance was set at $P<0.05$. F-tests were also undertaken to ensure equal variance between data samples at both 15 s and 45 s.

Confidence intervals, set at 95%, were calculated using the formula[13] 'Mean diff. ± (t-value x SE)'.These were calculated for each stage so that any inference made from the results of the subject group could be extrapolated to a larger population.

All statistical analysis was carried out using Microsoft Excel (MS Office XP).

Results

Relationships between 15 s and 45 s blood sampling time

Figure 1 shows a strong correlation between 15 s and 45 s sampling time for all subjects, whilst table 1 shows exact values for each stage and across all stages. The line of best fit and the identity line are very similar therefore the strength of the relation between the two variables and the agreement between them is high. A strong correlation was expected as both samples were taken from the same subject. However further agreement was sought using Limits of Agreement which would indicate how much the second blood sample agrees with the first. It can be seen in figure 2 that the range of the 95% LoA (-0.98 to 1.83) had a variance of 1.97 $mmol.l^{-1}$ across all 5 stages. This variance of 1.97 $mmol.l^{-1}$ would have more effect on a [Bla] of 1 $mmol.l^{-1}$ than [Bla] concentration of 9 $mmol.l^{-1}$ as proportionally 1.97 is a greater part of 1 $mmol.l^{-1}$ than it is of 9 $mmol.l^{-1}$. The data was then re-sorted so that results could be analysed by re-grouping into [Bla] concentration across the total 5 stages rather than stage by stage, the 15 second sampling time determining which group the results should be placed into. This would establish if the agreement between 15 s and 45 s sampling time showed higher agreement at a specific [Bla].

There was only 1 outlier across all concentrations when data were grouped by [Bla] concentration. All data fell within 1.5 $mmol.l^{-1}$ of the mean with the majority falling within a [Bla] difference of 0.75 $mmol.l^{-1.}$ The greatest fluctuations in the difference were observed between a [Bla] of 4 -7 $mmol.l^{-1}$. For each of the 5 stages and for the sum of all of the stages (figure 2), 95% of all [Bla] measurements at 15s and 45s sampling time fell between the mean±2SD. By further adjusting the difference to take into account imprecision at 1.5% for the Eppendorf [Bla] analyser the differences were less with the majority of the values lying within the mean±2SD moving values further within the limits of agreement. There was one outlier at Stage 2 and Stage 3.

A further relationship, looking at significance, was also examined and resulted in p values >0.05 using two-paired t-test two sample for means. An F-test had previously been performed for each stage and this indicated a low variance (P=>0.70) which provided validity for the use of t-tests assuming equal variance. The null hypothesis was therefore accepted suggesting that there was no significant difference between [Bla] collected at 15 s and 45 s (table 1).

To ensure that there was no significant difference between the two population's, confidence intervals for all stages were carried out at the 95% confidence level (table 1). All stages included zero suggesting that there was no significant difference between the two population groups.

Impact of Extension to Sampling Time

Lactate threshold curves were plotted against time for both 15 s and 45 s sampling times. This showed the similarity of the heart rate at threshold for both 15 s and 45 s sampling times and subsequent impact on training programmes based on HR training zones. Lactate threshold was taken as the point immediately preceding the curvilinear increase in blood lactate (inflection point) observed on the graph representing Lactate against Heart rate. These thresholds were verified independently by an experienced colleague not associated with the study, an example of which is shown in figure 3.

DISCUSSION

The major findings of this investigation were that an extension to blood sampling time does not significantly change [Bla] or change the lactate threshold at either of the sampling times. This indicates that there would be no negative effect on training programmes based on lactate threshold if sampling times were used interchangeably at different testing sessions.

From a review of published work[15,16,17,] the most prominent issues previously investigated are those describing metabolic pathways associated with [Bla], design of the protocol[18,19,20,21] sampling site[6,7] and those highlighting possible training effects on the [Bla] threshold.[1,18,22,23,24,25]

The majority of this work examined sampling sites, continuous versus discontinuous protocols and work stage duration and there was no information on the rationale for those blood sampling times selected. In the present study, the protocol used by the Olympic Medical Institute (OMI) and British International Rowing Organisation (BIRO) was examined. There was also no justification for blood sampling time of the OMI and BIRO protocol.

The main finding from the present study, that 95% of data fell within the Limits of Agreement for each of the five work stages, suggests a high agreement between the sampling times at 15s and 45. When data were grouped according to [Bla] there was only one outlier out of a possible eighty-five measured values. When all five work stages were grouped together (figure 2) there were only three values that fell outside the limits of agreement giving 97% agreement. The values that fell outside of the

Limits of Agreement and the two outliers from stages 2 and 3 may have been a remit of either non-compliance to the pre-test criteria or to measurement error.

There was no homogeneity of variance as the spread in the difference increased as [Bla] increased. This may be accounted for by the difference of 0.25 mmol.l^{-1} (0.25 being a nominal value for demonstration) within a 9 mmol.l^{-1} concentration which is proportionally much smaller than a 0.25 mmol.l^{-1} from 1 mmol.l^{-1} concentration, therefore a percentage change of 10% will be larger at the 9 mmol.l^{-1} (0.9%) concentration than at the (0.1%) 1 mmol.l^{-1} concentration giving a larger spread of difference as the intensity of the test increased.

There may have also been a greater difference between the subjects in the amount of [Bla] produced at higher exercise intensity and the rate of removal. This would be both as a result of training, allowing the athlete to become more efficient at [Bla] clearance, and also to the amount of Type I and Type IIa muscle fibres associated with each rower. Individual performance on a rowing ergometer was shown to be positively related to the rowers' ability to exchange and remove lactate[26]. Therefore inter-subject variation may have been greater at the higher [Bla] as more Bla is produced and each individual is able to clear Bla at a different rate. This source of variability could be reduced in future work by selecting subjects with similar [Bla] profiles. Although some variation in subjects' inflection point for blood lactate threshold was evident (figure 3), this produced no significant difference in heart rate used to predict training zones. Figure 3 also shows that below the lactate threshold the values at 45 s are lower than those at 15 s and above the lactate threshold the opposite can be seen. This would not be unusual, as below the lactate threshold the extension to sampling time allows for net clearance of Bla but above the lactate threshold Bla would be appearing in high concentrations throughout the full 45 s interval. From sixteen individualised lactate thresholds, 11 had lower [Bla] at the 45 s sampling time below threshold and 5 had [Bla] higher at 45 s below threshold. Above threshold there was no clear pattern.

Further, figure 2 shows that below the 2 mmol.l^{-1} level of Bla accumulation, which represents the average lactate threshold for the subjects, Bla clearance may have been greater than Bla accumulation as the subjects were able to clear Bla efficiently at lower levels of exercise intensity. It is important to note that this was not shown when the threshold for the onset of blood lactate accumulation (OBLA) was represented by the standard value of 4 mmol.l^{-1}. Once the onset of Bla accumulation has been reached the subject will experience a decline in [Bla] clearance and [Bla] accumulation will be seen.[3,16,26]. At stage 5 the majority of change between 15 s and 45 s shifted from a negative to a positive difference. Those subjects who did not show this positive difference were better able to cope with [Bla] clearance due to training adaptations and/or being physiologically advantaged in this way. Observing data grouped into sub-sets of [Bla] concentration (as opposed to stages across the rowing test) there was much less of a difference from the mean at the lowest [Bla] and a much greater spread at the higher [Bla]. This may be attributed to lower exercise intensities producing less [Bla]; therefore there was less to be cleared whereas at the higher intensities efficiency of [Bla] clearance may have an effect on the difference from the mean. By grouping data into [Bla], fluctuations were seen relative to the [Bla], whereas observing the data across each of the five stages, subjects with higher aerobic fitness and enhanced ability to clear lactate would produce lower blood [Bla]

at each stage and may not show as great a change in [Bla] at each level as the less highly trained athletes, as they were used to working intensely and did not produce high [Bla] until a higher workload is reached.[18] Although each subject was given an individual workload relative to their personal best time the subject's fitness level would still influence the level that could be worked at before [Bla] accumulation becomes greater than [Bla] clearance.

Confidence intervals were undertaken so that a range of values surrounding the sample, within which, at a given level of confidence (95%), the true value of the corresponding parameter in the population is likely to be found.[14] The confidence intervals gave a much smaller range for the differences than those of the limits of agreement and allowed in some cases less than 0.05 $mmol.l^{-1}$ change from the mean. The size of the critical t value (t=2.12) multiplied by the standard error would account for these small intervals. However when the confidence intervals were applied to the data the difference from the lower limit to the upper limit varied between 1.36 to 1.96 $mmol.l^{-1}$ across the stages. It would be reasonable to expect the change between the 15 s sampling time and 45 s sampling time to fall within these parameters. Confidence intervals for all five stages include zero, there was therefore no significant difference between the two populations at the 95% Confidence Level.

In Bland & Altman's original paper[13], Limits of Agreement were used to show agreement between two sets of figures where one would expect there to be no change between the actual data but a difference between the readings of one piece of equipment used to analyse the results against another piece of equipment. Therefore both readings could be identical. However, in this investigation a change between the two sets of data would be expected within a 30 s period as the normal physiological process of [Bla] accumulation or removal would change the concentration of [Bla]. Even when taking this into consideration 95% limits of agreement were obtained on all data.

This implies that there was a high association between the two sampling times. The limitation of using a correlation co-efficient is that it only highlights a relationship and association and not agreement. A high correlation would be expected between the two blood [Bla] samples as they have each come from the same subject undertaking the same stress test at the same time. A strong relationship was demonstrated but this does not show whether the values have agreement or are significantly different. For example, if all 45 s samples were consistently 50% above the 15 s sample, this would show a high correlation but would not show agreement or allow the investigator to determine whether the two sampling times could be used interchangably.

Practical Applications

The specific aim for this investigation was to examine the validity of extending the current sampling time for blood collection when rowing. The present measurement interval currently used by the OMI and BIRO is a maximum of 30 s, which is adequate when testing is performed indoors on a rowing ergometer, however this time

can limit the practicality of testing in a boat on the water. The above investigation was performed to identify if blood sampling time could be extended to 45 s without negatively impacting on training programmes based on these results. This could theoretically be applicable for blood sampling when testing on the water thus allowing comparison of blood samples collected across a range from 15 s to 45 s providing a total collection period of 30 s. There was no significant difference between the two sampling times and a strong agreement between the data. We therefore conclude that this provides justification for extending the current maximal sampling time from 30s to 45 s.

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Table 1. P values and Pearson correlation co-efficient for the relationship between [Bla] at 15 s and 45 s for each stage of a five stage incremental rowing test and across all five stages and 95% confidence intervals for [Bla] at 15s and 45s for each of the five stages.

Stage of Test	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**	**All Stages**
Correlation	0.92	0.79	0.94	0.93	0.97	0.97
P Value (2 tail)	0.597	0.602	0.806	0.396	0.089	0.098
CI (95%)	-0.500 to 0.051	- 0.767 to 1.129	-0.728 to 0.840	-0.900 to 1.346	-0.577 to 1.543	-------

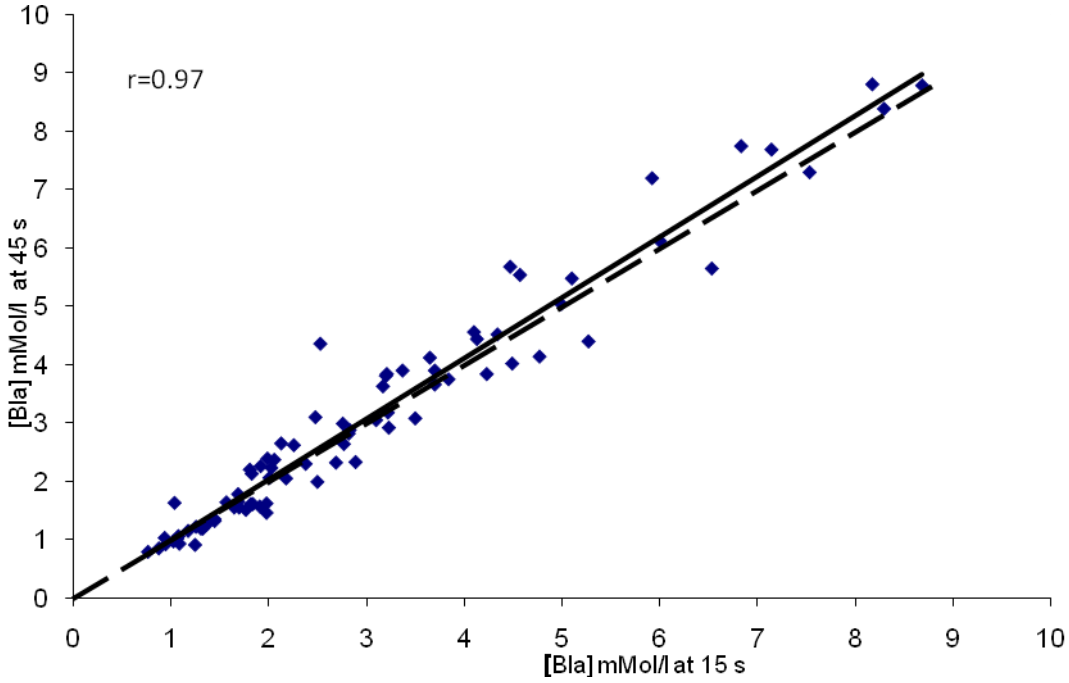

Figure 1. 15s and 45s sampling time to show correlation between [Bla]across all 5 stages of the test. Dashed line is the identity line (x=y).

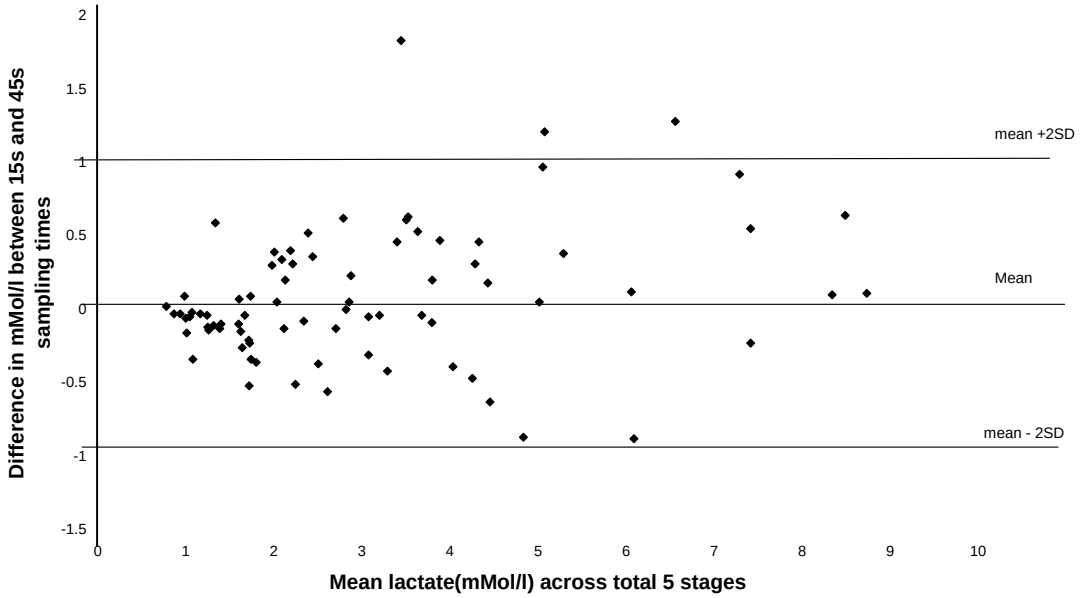

Figure 2. 95% Limits of Agreement for [Bla] at 15 s and 45 s across all stages of an incremental rowing test.

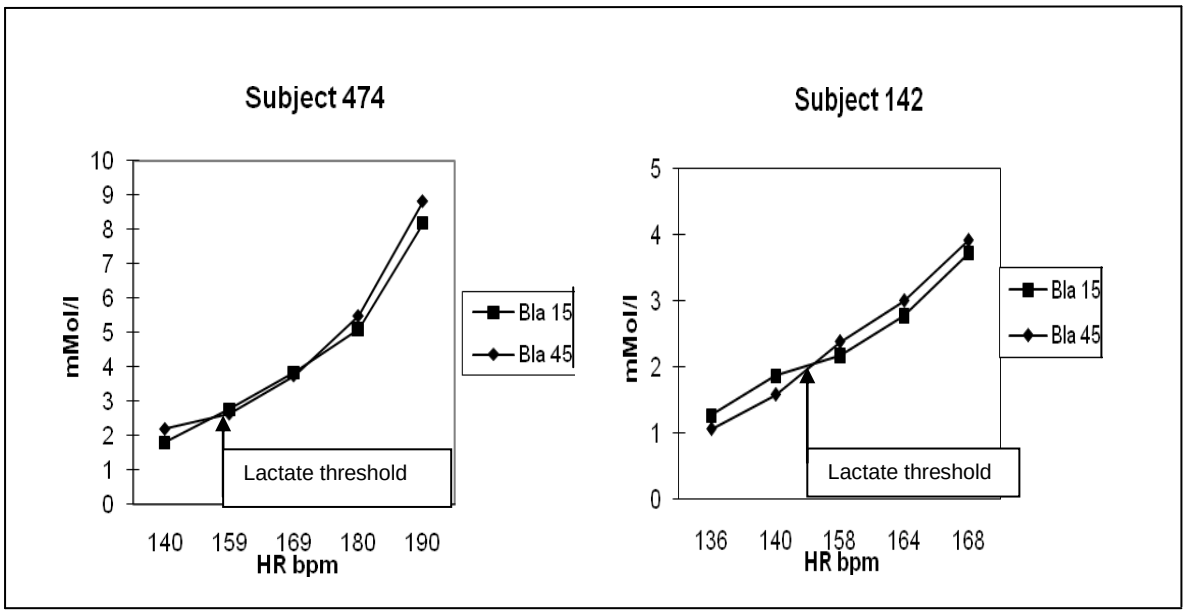

Figure 3 : Lactate Vs heart rate curves for both 15 and 45 s sampling times.